Box SEQ 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
Patent Examining Operations

Applicant(s): Leslie S. Johnson

Serial No: Art Unit: Unassigned

Filed: September 21, 1998 Examiner: Unassigned

Title: Human-Murine Chimeric Antibodies Against Respiratory Syncytial Virus

Attorney Docket No.: 469201-367

TRANSMITTAL LETTER

Assistant Commissioner for Patents
Washington, D.C. 20231

SIR:

Enclosed please find the following:

1. Request for Filing a Divisional Application;
2. Copy of prior application Serial No. 08/290,592 as filed;
3. Copy of executed Declaration and Power of Attorney;
4. Preliminary Amendment;
5. Corrected Figure 7;
6. Sequence Listing;
7. Computer Disk;
8. Check No. 27721 in the amount of $872.00; and
9. A self-addressed, postage paid, return receipt postcard, date stamp and return of which is respectfully requested.

The Commissioner is authorized to charge payment of any additional filing fees required under 37 C.F.R. 1.16 associated with this communication or credit any overpayment to Deposit **Account No. 03-0678**.

EXPRESS MAIL CERTIFICATE

Express Mail Label No. EM471209787US

Deposit Date: September 21, 1998

I hereby certify that this paper and the attachments hereto are being deposited today with the U.S. Postal Service "Express Mail Post Office To Addressee" service under 37 CFR 1.10 on the date indicated above addressed to:

BOX CONTINUATION APPLICATION

Assistant Commissioner for Patents

Washington, DC 20231

Elliot M. Olstein 9/21/98

Elliot M. Olstein, Esq. Date

Respectfully submitted,

Elliot M. Olstein

Elliot M. Olstein, Esq.
Reg. No. 24,025
CARELLA, BYRNE BAIN, GILFILLAN,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Leslie S. Johnson		
Divisional of Serial No.:	08/290,592	Group:	Unassigned
Parent Filed:	August 15, 1994	Examiner:	Unassigned
For:	Human-Murine Chimeric Antibodies Against Respiratory Syncytial Virus		
Docket No.:	469201-367		

Honorable Assistant Commissioner for Patents
Box Continuation Application
Washington, D.C. 20231

Sir:

This is a request for filing a:

_____ Continuation ___XX___ Divisional

Application under 37 CFR 1.53, of pending prior application

Serial No. 08/290,592 filed on August 15, 1994

of Leslie S. Johnson
(Applicant)

for HUMAN-MURINE CHIMERIC ANTIBODIES AGAINST RESPIRATORY SYNCYTIAL VIRUS
(Title)

1. X Enclosed is a copy of the prior application (28 pages of specification, four pages of claims, a one-page abstract, and 10 sheets of drawings) including the oath or declaration as originally filed and an affidavit or declaration verifying it as a true copy.

2. X The filing fee is calculated below:

CLAIMS AS FILED, LESS ANY CLAIMS CANCELLED BY AMENDMENT

For	Number filed	Number extra	Rate	Basic Fee
				$790.00
Total Claims	10 - 20=	0	$11.00 [ ] 22.00 [ ]	
Independent Claims	4 - 3=	1	$41.00 [ ] 82.00 [X]	$82.00
		TOTAL FILING FEE $		$872.00

3. _X_ The Commissioner is hereby authorized to charge the above fee and any fees which may be required, or to credit any overpayment to Deposit Account No. 03-0678. A duplicate copy of this sheet is enclosed.
4. _X_ A check in the amount of $872.00 is enclosed.
5. _X_ Cancel in this application original claims 5, 8, 9, 11, 12, and 14-20 of the prior application before calculating the filing fee and insert new claims 21 and 22 from the preliminary amendment appended hereto.
6. _X_ Amend the specification by inserting before the first line the sentence:
   --This is a Divisional of Application Serial No. 08/290,592 filed August 15, 1994 which is a continuation-in-part of Application Serial No. 07/813,372, filed December 23, 1991, abandoned.--
7. _X_ Also appended are copies of the originally filed drawings (10 sheets, Figures 1-10) as filed with the prior application.
8. __ New formal drawings as filed in the parent application are enclosed. Accordingly please delete the original and insert the enclosed formal drawings.
9. __ The certified copy has been filed in prior application Serial No. _____ filed _____.
10. _X_ The prior application is assigned of record to:
    MedImmune, Inc., 35 West Watkins Mill Road, Gaithersburg, MD 20878.

11. X The power of attorney in the prior application is to Elliot M. Olstein (Reg. No. 24,025) of Carella et al., 6 Becker Farm Road, Roseland, NJ 07068-1739.
    (a) ___ The Power appears in the original papers of the prior application.
    (b) X Since the Power does not appear in the original papers, a copy of the power in the prior application is enclosed.
    (c) X Address all future communication to: Elliot M. Olstein at the above address.
12. ___ A Preliminary Amendment will be filed at a later date.
13. X I hereby verify that the attached papers are a true copy of the prior application Serial No. 08/290,592 as originally filed on August 15, 1994.
14. ___ Verified Statement Claiming Small Entity is enclosed.

The undersigned declares further that any statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

EXPRESS MAIL CERTIFICATE

Express Mail Label No. EM471209787US

Deposit date: September 21, 1998

I hereby certify that this paper and the attachments hereto are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above addressed to:
Box Continuation Application
Assistant Commissioner for Patents
Washington, DC 20231

Elliot M. Olstein 9/21/98

Elliot M. Olstein, Esq. **Date**

Respectfully submitted,

Elliot M. Olstein

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of	:	Johnson
Serial No.	:	
Filed	:	
For	:	Human–Murine Chimeric Antibodies Against Respiratory Syncytial Virus
Group	:	Unassigned
Examiner	:	Unassigned

Assistant Commissioner of Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

As a Preliminary Amendment to the above-identified application, kindly amend the above-identified application as follows:

IN THE SPECIFICATION:

At Page 3, line 29, after "grafting," insert --(SEQ ID NO:16)--.

At Page 3, line 29, after "grafted V_H," insert --(SEQ ID NO:17)--.

At Page 3, line 30, after "Mab 1308F V_H," insert --(SEQ ID NO:18)--.

At Page 4, line 5, after "grafting," insert --(SEQ ID NO:19)--.

At Page 4, line 5, after "grafted V_L," insert --(SEQ ID NO:20)--.

At Page 4, line 6, after "MAb 1308F V_L," insert --(SEQ ID NO:21)--.

At Page 4, line 12, after "sequences," insert --(SEQ ID NO:22) through (SEQ ID NO:25)--.

At Page 4, line 15, after "sequences," insert --(SEQ ID NO:26) through (SEQ ID NO:29)--.

At Page 4, line 23, after "grafting," insert --(SEQ ID NO:30)--.

At Page 4, line 23, after "grafted V_H," insert --(SEQ ID NO:31)--.

At Page 4, line 24, after "Mab 1129V_H," insert --(SEQ ID NO:32)--.

At Page 4, line 30, after "grafting," insert --(SEQ ID NO:33)--.

At Page 4, line 30, after "grafted V_L," insert --(SEQ ID NO:34)--.

At Page 4, line 31, after "Mab 1129V_L," insert --(SEQ ID NO:35)--.

At Page 10, line 7, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:1)--.

At Page 10, line 9, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:2)--.

At Page 10, line 27, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:3)--.

At Page 10, line 28, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:4)--.

At Page 16, line 24, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:1)--.

At Page 16, line 26, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:5)--.

At Page 16, line 28, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:6)--.

At Page 16, line 30, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:7)--.

At Page 16, line 32, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:8)--.

At Page 17, line 2, after the oligonucleotide sequence set forth, insert --(SEQ ID NO:9)--.

At Page 18, line 18, after "(Figure 9)," insert --(SEQ ID NO:36) through (SEQ ID NO:42)--.

Kindly insert the accompanying sequence listing between the specification and claims.

Kindly replace Figure 7 with the corrected Figure 7 accompanying this preliminary amendment.

IN THE CLAIMS:

Cancel Claims 5, 8, 9, 11, 12, and 14-20 without prejudice.

Amend the following claims:

1. (Amended) A human-murine [chimeric] antibody against respiratory syncytial virus, comprising:

a human antibody containing at least one CDR from each of the variable heavy and variable light chains of a [non-human] murine monoclonal antibody against [RSV] respiratory syncytial virus.

2. (Amended) [An] The antibody [as in] of Claim [1,] 21 wherein said murine monoclonal antibody is a neutralizing antibody against [RSV] respiratory syncytial virus.

3. (Amended) [An] The antibody [as in] of Claim [1,] 21 wherein said murine monoclonal antibody is an antibody against [RSV] respiratory syncytial virus F protein.

4. (Amended) [An] The antibody [as in] of Claim 3[,] wherein said murine monoclonal antibody is a neutralizing antibody against [RSV] respiratory syncytial virus F protein.

6. (Amended) [An] The antibody of Claim [5] 3 wherein said murine antibody against [RSV] respiratory syncytial virus F protein is specific for antigenic site A of said protein.

7. (Amended) [A human] The antibody of Claim [5] 3 wherein said murine antibody against [RSV] respiratory syncytial virus F protein is specific for antigenic site C of said protein.

ADD THE FOLLOWING CLAIMS:

21. An antibody against respiratory syncytial virus, comprising:

a human constant region, a heavy chain and light chain variable region, each of which comprises a framework region, at least a portion of which is human, and three complementarity determining regions, each complementarity determining region being derived from a murine monoclonal antibody.

22. A process for preventing or treating a respiratory syncytial virus infection in an animal, comprising:

administering to said animal an effective amount of the antibody of Claim 21.

REMARKS

The above is a preliminary amendment to the divisional application of application Serial No. 08/290,592.

A sequence listing in paper and in computer readable form accompanies this preliminary amendment. Pursuant to 37 CFR 1.821(f), please be advised that the paper and computer readable copies of the sequence listing are the same. The specification has been amended to provide references to the sequence identification numbers. Claims 5, 8, 9, 11 12 and 14-20 have been cancelled without prejudice, and Claims 21 and 22 have been added.

It is respectfully requested that the above preliminary amendment be entered, and an early notice of allowance is hereby solicited.

Respectfully submitted,

Elliot M. Olstein

Elliot M. Olstein
Registration No. 24,025

9/21/98

W:\PATENTS\Amendments\Application of Johnson

P:\OP\PATAP166

Human-Murine Chimeric Antibodies Against Respiratory Syncytial Virus

BACKGROUND

This application is a continuation-in-part of U.S. Application Serial No. 07/813,372, filed on December 23, 1991.

Respiratory syncytial virus (RSV) is the major cause of acute respiratory illness in young children admitted to hospitals, and the community practice will treat perhaps five times the number of hospitalized children. It is therefore, the most common cause of lower respiratory tract infection in young children. While the majority of community-acquired RSV infections resolve themselves in a week to ten days, many hospitalized children, especially under six months of age require assisted ventilation.

Efforts to produce an effective vaccine have been unsuccessful (8). A major obstacle to vaccine development is safety; the initial formalin inactivated RSV vaccine caused an increased incidence of RSV lower respiratory tract disease and death in immunized children upon exposure to virus (5).

Recently, the drug ribavirin has been licensed for therapy of RSV pneumonia and bronchiolitis (2,3); its value is contraversial (4). Although ribavirin has shown efficacy (9), the drug has to be

administered over an 18 hour period by aerosol inhalation. In addition, the level of secondary infections following cessation of treatment is significantly higher than in untreated patients.

Studies have shown that high-titered RSV immunoglobulin was effective both in prophylaxis and therapy for RSV infections in animal models (6, 7). Infected animals treated with RSV immune globulin, showed no evidence of pulmonary immune-complex disease (6, 7).

Even if RSV hyperimmune globulin is shown to reduce the incidence and severity of RSV lower respiratory tract infection in high risk children, several disadvantages may limit its use. One drawback is the necessity for intravenous infusion in these children who have limited venous access because of prior intensive therapy. A second disadvantage is the large volume of RSVIG required for protection, particularly since most these children have compromised cardiopulmonary function. A third disadvantage is that intravenous infusion necessitates monthly hospital visits during the RSV season which places these children at risk of nosocomial RSV infection (1). A final problem is that it may prove to be very difficult to select sufficient donors to produce a hyperimmune globulin for RSV to meet the demand for this product. Currently only about 8% of normal donors have RSV neutralizing antibody titers high enough to qualify for the production of hyperimmune globulin.

Another approach may be the development of monoclonal antibodies with high specific neutralizing activity as an alternative to hyperimmune globulin. It is preferable, if not necessary, to use human monoclonal antibodies rather than murine or rat antibodies to minimize the development of human anti-rodent antibody responses which may compromise the therapeutic efficacy of the antibody or induce immune-complex pathology. However, the generation of human monoclonal antibodies with the desired specificity may be difficult and the level of production from human cell lines is often low, precluding their development.

An alternative approach involves the production of human-mouse chimeric antibodies in which the genetic information encoding the murine heavy and light chain variable regions are fixed to genes encoding the human heavy and light constant regions. The resulting mouse-human hybrid has about 30% of the intact immunoglobulin derived from murine sequences. Therefore, although a number of laboratories have constructed chimeric antibodies with mouse variable and human constant domains (10-18), the mouse variable region may still be seen as foreign (19).

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a complementarity determining region (CDR)-grafted human antibody which contains at least one CDR from each variable heavy chain and variable light chain of at least one monoclonal antibody against the RSV antigen. The monoclonal antibody may be derived from any non-human animal, preferably howver, it is derived from a rodent and most preferably it is a murine monoclonal antibody. Preferably, the murine monoclonal antibody is a neutralizing antibody. It is also preferable that said murine antibody is an antibody against RSV F antigen.

The term "animal" as used herein is used in its broadest sense includes mammals including humans.

DETAILED DESCRIPTION OF THE DRAWINGS

The drawings depicted and described herein are intended to further illustrate the present invention and are not intended to limit the invention in any manner whatsoever.

Figure 1 shows the amino acid (AA) sequence design of CDR-Grafted anti-RSV F glycoprotein V_H. The figure depicts the AA sequence for the human HV3 V_H before grafting, CDR grafted V_H, and murine MAb1308F V_H from which the CDR sequence was grafted. The heavily underlined regions identify the CDR sequence which was

grafted into the human HV3 V_H and each of the three regions is identified as CDR1, CDR2 and CDR3, respectively.

Figure 2 shows the amino acid (AA) sequence design of CDR-Grafted anti-RSV F Protein V_L. The figure depicts the AA sequence for the human K102 V_L before grafting, CDR grafted V_L, and murine MAb1308F V_L from which the CDR sequence was grafted. The heavily underlined regions identify the CDR sequence which was grafted into the human K102 V_L and each of the three regions is identified as CDR1, CDR2 and CDR3, respectively.

Figure 3 depicts the oligonucleotides used to make Hu1308V_H, the sequences which are underlined are the specific primer sequences.

Figure 4 depicts the oligonucleotides used to make Hu1308V_L, the sequences which are underlined are the specific primer sequences.

Figure 5 depicts the plasmid construction of the expression vectors for Humanized 1308.

Figure 6 depicts a graph of the Neutraliziation of RSV as percent neutralization versus ng MAb per reaction for neutralizing with Cos Hu1308F and with Mu1308F.

Figure 7 shows the amino acid (AA) sequence design of CDR-Grafted anti-RSV F glycoprotein V_H. The figure depicts the AA sequence for the human COR V_H before grafting, CDR grafted V_H, and murine MAb1129 V_H from which the CDR sequence was grafted. The heavily underlined regions identify the CDR sequence which was grafted into the human COR V_H and each of the three regions is identified as CDR1, CDR2 and CDR3, respectively.

Figure 8 shows the amino acid (AA) sequence design of CDR-Grafted anti-RSV F Protein V_L. The figure depicts the AA sequence for the human K102 V_L before grafting, CDR grafted V_L, and murine MAb1129 V_L from which the CDR sequence was grafted. The heavily underlined regions identify the CDR sequence which was grafted into the human K102 V_L and each of the three regions is identified as CDR1, CDR2 and CDR3, respectively.

Figure 9 shows the oligonucleotides used to construct the humanized 1129 VH.

Figure 10 shows binding data for humanized 1129 in an ELISA assay.

DETAILED DESCRIPTION OF THE INVENTION

Applicants have found that transplantation into a human antibody, of only the genetic information for at least one CDR from each of the variable heavy and variable light chain derived from murine monoclonal antibody against RSV antigen, is effective for the prevention and treatment of RSV in animals. Preferably the murine antibody is a neutralizing antibody against RSV. Another aspect of the present invention provides for the murine antibody to be an antibody against RSV F antigen. Preferably, the murine antibody is neutralizing antibody against RSV F antigen. The substitution of the mouse CDR's into the human variable framework segments minimizes the potential for human anti-mouse antibody (HAMA) responses while retaining binding affinity and specificity for antigen, RSV F protein. Since, the CDR's do not contain characteristic murine or human motifs, the human antibodies containing the murine antibody CDR's are essentially indistinguishable from completely human antibodies, thereby, minimizing the human antibody response while retaining binding affinity and specificity for RSV F antigen.

The development of a humanized antibody against RSV F antigen began with a murine antibody against RSV F antigen. Examples of murine antibodies of this type are: MAb 1436C, MAb 113, MAb 112, MAb 151, MAb 1200, MAb 1214, MAb 1237, MAb 1129, MAb 1121, MAb 1107, MAb 131-1, MAb 43-1, MAb 1112, MAb 1269, MAb 1243, MAb 1331H, MAb 1308F and MAb 1302A (see citation 21).

An aspect of the present invention provides that the CDRs of the human antibody are comprised of three complementarity determining regions (CDRs) from each variable heavy and variable light chain of the murine antibody.

The murine antibodies against RSV F antigen have been mapped by competitive binding and reactivity profiles of virus escape mutants to three broad antigenic sites (A, B, C) containing 16 distinct epitopes (20). The epitopes within antigenic sites A and C have shown the least variability in natural isolates.

Therefore, another aspect of this invention provides for a human antibody containing at least one CDR from each variable heavy and variable light chain of at least one murine antibody against RSV F antigen which is specific for antigenic site A or C. In one aspect, this invention provides for the murine antibody against RSV F antigen specific for antigenic site C, where the murine antibody is MAb 1308F.

In such an embodiment of this invention a human antibody contains CDR's of the variable heavy chain of murine antibody MAb 1308F against the RSV F antigen. The CDR variable heavy chain of MAb 1308F comprises three CDRs having the following amino acid sequences: Nos. 31 to 35, 47 to 60 and 99 to 106. In addition, this embodiment contains CDR's of a variable light chain of MAb 1308F of murine antibody against RSV F antigen. The CDR variable light chain comprises three CDR's having the following amino acid sequences: Nos. 24 to 34, 50 to 56 and 89 to 97.

Another aspect of this invention provides for a human antibody containing at least one CDR from each variable heavy and variable light chain of at least one murine antibody against RSV F antigen which is specific for antigenic site C. Preferably, this invention provides for the murine antibody against RSV F antigen specific for antigenic site C, where the murine antibody is MAb 1129.

In the embodiment of this invention a human antibody which contains CDR's of the variable heavy chain of murine antibody MAb 1129 against the RSV F antigen. The CDR variable heavy chain of MAb 1129 comprises three CDRs having the following amino acid sequences: Nos. 31 to 36, 52 to 67 and 100 to 109. In addition, this embodiment contains CDR's of a variable light chain of MAb 1129 of murine antibody against RSV F antigen. The CDR variable

light chain comprises three CDR's having the following amino acid sequences: Nos. 24 to 33, 51 to 56 and 89 to 96.

An additional aspect of applicants' invention is a process for preventing or treating RSV infection comprising administering to the animal an effective amount of a human antibody containing at least one CDR from each variable heavy and variable light chain, of at least one murine antibody against RSV F antigen.

Another aspect of applicants' invention is a composition comprising administering an effective amount of the human antibody as described above in conjunction with an acceptable pharmaceutical carrier. Acceptable pharmaceutical carriers include but are not limited to non-toxic buffers, fillers, isotonic solutions, etc.

The composition of Applicant's invention may be administered topically or systemically. Examples of topical administration are intranasal administration and inhalation of an aerosol containing the human antibody composition. Systemic administration may be accomplished by intravenous or intramuscular injection of the human antibody composition.

A preferred aspect of Applicants' invention is that the human antibody is administered as part of a plurality of human antibodies against RSV F antigen. These antibodies can be against the same or different epitopes of the RSV F antigen.

Additionally, the human antibody of this invention can be used clinically for diagnosing respiratory syncytial virus in patients. Because of their affinity for RSV F antigen these human antibodies can be used in known diagnostic assay procedures for detecting the presence and concentration of RSV F antigen cells in samples, e.g., body fluids. The human antibodies of the present invention can for example be attached or bound to a solid support, such as latex beads, a column, etc., which are then contacted with a sample believed to contain RSV F antigen.

Applicants' development of human antibodies against RSV, began with murine hybridoma cells producing murine monoclonal antibodies

which have been shown to neutralize RSV in vitro and protect cotton rats against lower respiratory tract infection with RSV.

One such antibody was selected, which is specific for antigenic site C, to produce mouse-human chimeric antibodies. This antibody was chosen on the basis that it: (i) reacted with a large number of virus strains tested (at least 13 out of 14 isolated); (ii) retained neutralizing activity against virus escape mutants selected with other anti-F antibodies and (iii) blocked RSV replication when administered at low doses to cotton rats by intranasal route prior to virus challenge. The antibody showed significant reduction in pulmonary virus titer among antibodies in that respective region. Murine antibody 1308F, specific for the C region of RSV F protein, was chosen as the initial target for humanization.

In summary, the human antibodies were constructed as follows: the RNA was extracted from the murine antibody-producing cell line, the murine variable regions which are responsible for the binding of the antibody to RSV were cloned and sequenced, resulting in the identification of the murine antibody CDRs. Then a human variable heavy and light chain framework sequence having the highest homology with the variable heavy and light chain murine antibody, was selected. A human framework sequence such as described above is best able to accept the murine-derived CDRs.

The murine 1308F variable heavy chain was compared to various human germline genes, the highest homology was to the human germline gene HV3. The two sequences were 62% homologous overall and 65% in the framework regions. Significantly, there is good homology at the junctions of the CDR segments and the frameworks with the exception of the 5′ end of FR2. The murine derived variable heavy chain CDRs were then substituted into the variable heavy chain human germline gene HV3. The mouse and human sequences as well as that of a potential CDR-Grafted combination of the two is shown in Figure 1.

A similar analysis of the V_L region revealed high homology to the human germ line V-Kappa gene K 102. The alignment of these sequences is shown in Figure 2. In this case the homology is 62% overall and 73% in the framework regions. The murine-derived variable light CDRs were then substituted into the human variable light chain of human germline gene K102.In each case a human J-region can be selected which is identical to the mouse sequence.

In another embodiment, murine 1129 variable heavy chain was compared to various human variable region amino acid sequences, the highest homology was to the human rearranged COR sequence. The two amino acid sequences were 75% homologous overall and 80% in the framework regions. Significantly, there is good homology at the junctions of the CDR segments and the frameworks. The murine derived variable heavy chain CDRs were then substituted into the variable heavy chain human COR V_H sequence. The mouse and human sequences as well as that of a potential CDR-Grafted combination of the two is shown in Figure 1.

A similar analysis of the V_L region revealed high homology to the human germ line K102. The alignment of these sequences is shown in Figure 8. In this case the homology is 73% overall and 82% in the framework regions. The murine-derived variable light CDRs were then substituted into the human variable light chain of human germline K102. In this case a human J-region, human JK4, was selected which is similar to the mouse sequence.

Therefore, human antibodies are expressed and characterized relative to the parental murine antibodies to be certain that the genetic manipulation has not drastically altered the binding properties of the antibodies.

Applicants present herein examples which are further illustrative of the claimed invention but not intended to limit the invention.

Examples 1

cDNA cloning and sequencing of anti-RSV F Protein antibody 1308F

cDNA copies of the V_H and V_L of the target antibody were generated as follows. The first strand CDNA reaction was carried out using AMV reverse trenscriptase and a phosphorylated oligonucleotide primer complementary to a segment of the mRNA coding for the constant region of the particular heavy or light chain isotype. For 1308F the isotype is gammal, kappa and the specific oligonucleotides were 5'AGCGGATCCAGGGGCCAGTGGATAGAC complementary to codons 129-137 of the CH1 region of the murine Gammal gene, and 5'TGGATGGTGGGAAGATG complementary to codons 116-122 of the murine C-kappa gene. The primer anneals to a segment of the mRNA adjacent to the variable region. Second strand cDNA synthesis was carried out using RNase H and E. coli DNA polymerase I, as described by Gubler and Hoffman (Gene 25,;263, 1983), followed by T4 DNA polymerase to assure that blunt ends are produced.

Signal	V	J	C	mRNA
	1st strand cDNA			
	2nd strand cDNA			

The ds-cDNA was ligated into pUC18 which had been digested with restriction endonuclease SmaI and treated with alkaline phosphatase. The ligation was used to transform E. coli DH5a by the method of Hanahan (J. Mol. Biol. 166;557, 1983). Oligonucleotide probes corresponding to C-region sequence lying between the first strand cDNA primer and the V-region were used in colony hybridizations to identify transformants carrying the desired cDNA segment. The specific probe sequences were GGCCAGTGGATAGAC complementary to codons 121-125 of murine CH1 regions and TACAGTTGGTGCAGCA complementary to codons 110-115 of c-Kappa, respectively. Candidate plasmids, isolated from colonies which were positive in the hybridization, were analyzed by

digestion with restriction endonucleases Eco RI and Hind III to release the cDNA insert. Those with inserts of 400-500bp were subjected to DNA sequencing.

The cDNA inserts were inserted into M13 mp18 and mp19 for the determination of the DNA sequence on both strands. Single stranded DNA from the resulting recombinant bacteriophage was isolated and sequenced by the dideoxy chain termination method (Proc. Nat. Acad. Sci. USA 74; 5463, 1977).

In order to confirm that the pair of rearranged and somatically mutated V gene cDNA's isolated from the 1308F hybridoma represented those which were in the 1308F antibody, a single-chain Fv gene was generated, expressed in and secreted from mammalian cells, then assayed for binding to RS virus. Competition binding experiments then were used to demonstrate the identity of the binding site.

Example 2

Design and assembly of human 1308F V_H and V_L

The CDR regions of the V_H and V_L were identified by comparing the amino acid sequence to known sequences as described by Kabat (38). In order to select the human framework sequences best able to accept the mouse derived CDR sequences in a conformation which retains the structure of the antigen combining site, the following strategy was employed. First, the sequence of the murine V_H and V_L regions will be compared to known human sequences from both the Genbank and NBRF protein databanks using the Wordsearch program in the Wisconsin package of sequence manipulation programs (Nucleic Acid Res. 12;387). The best several human V-regions were then analyzed further on the basis of similarity in the framework regions, especially at the junctions of the framework and CDR regions (see Figures 1 and 2).

The CDR-grafted V_H region together with the respective leader sequence of the human v-region gene was synthesized de novo using four overlapping oligonucleotides ranging from 100-137 nucleotides

in length (see Figure 3). The oligonucleotides were first allowed to anneal in pairwise combinations and extended with DNA polymerase to generate approximately 200bp ds DNA fragments with an overlapping region. the fragments were then mixed and subjected to PCR using primers at the 3'end of one fragment and the 5' end of the other fragment. The only product which can be formed under these condition is the full length V_H segment. The specific primer sequences are underlined in Figure 3. An endonuclease Sac I site was included at the 3' end of the V_H sequence in order to join it to a human constant region gene segment.

The CDR-grafted V_L region was synthesized in a similar way (see Figure 4). In this instance the initial 200bp fragments were amplified separately and inserted into separate plasmeds. The fragment coding for the amino terminus was cloned into a pUC18 derivative as an NcoI-SmaI fragment while the fragment coding for the carboxyl-terminus was cloned as a SmaI to Hind III fragment. The fragments were subsequently combined via a SmaI site at the junction. The oligonucleotides are indicated in Figure 4. A Hind III site was included near the 3' end of the gene segment in order to join it to a human C-kappa gene.

Example 3

Construction of Vectors for 1308F expression

The NcoI-SacI fragment representing the humanized V_H was joined to a SacI -Notl fragment representing a human c-Gamma I CDNA and inserted into pS 18 (which is pUC 1 8 with Ncol and NotI restriction sites incorporated into the polylinker region between the BamHI and Kpnl sites). The humanized 1308F-gammal gene on a SacI-NotI fragment was then combined with a Pvul-NotI fragment from pSJ37 carrying a poly A addition site and a PvuI-SacI fragment from pSV2-dhfr-pCMV containing the SV40 origin of replication, a dhfr gene and the CMV immediate early promoter. The resulting plasmid was designated pSJ60.

The NcoI-HindIII fragment representing the humanized V_L was joined to a HindIII-NotI fragment representing a human c-Kappa CDNA in pS18. The humanized 1308F-Kappa gene on a SalI-NotI fragment was then combined with a PvuI-NotI fragment from pSJ37 carrying a poly A addition site and a PvuI-SalI fragment from pSV2-dhfr-pCMV, containing the SV40 origin of replication, a dhfr gene and the CMV immediate early promoter. The resulting plasmid was designated pSJ61.

Finally pSJ60 and pSJ61 were combined into a single plasmid containing both the light and heavy chains and expression signals. This was accomplished by isolating a PvuI-Bam HI fragment from pSJ61 carrying the light chain with a Pvu I - Bgl II fragment from pSJ60 carrying the heavy chain to generate pSJ66. (See Figure 5).

Example 4

Transfection of Cosl cells with PSJ60 and PSJ61

Transfections were carried out according to the method of McCutchan and Pagano (J. Nat. Can. Inst. 41: 351-356, 1968) with the following modifications. COS 1 cells (ATCC CRL1650) were maintained in a humidified 5% C02 incubator in 75 cm^2 tissue culture flasks in Dulbecco's Modified Eagle Medium (DMEM, GIBCO #320-1965) supplemented with 10% Fetal Bovine Serum (FBS, GIBCO #200-6140) and 2mM L-glutamine (BRL #320-5030) and passed at a split ratio of 1:20 when the cells had reached confluence. 48 hours prior to transfection, 5 100mm tissue culture dishes were seeded with 1.5 x 10^6 cells per dish in 12ml DMEM, 10% FBS, 2mM L-glutamine, 1% penicillin-streptomycin (P-S, GIBCO #600-5070). The day of the transfection, 120 ug each of the plasmids pSJ60 and pSJ61 were combined, ethanol precipitated, and aseptically resuspended in 2.5ml Tris-Buffered-Saline. The resuspended DNA was added dropwise, with mixing, to 10ml of DMLEM containing 1 mg/ml DEAE-dextran (Phamiacia #17-0350-01) and 250 uM chloroquine (Sigma #C6628). The medium was removed from the COS1 cells in the 100 mm dishes and the cells were washed once with Dulbecco's phosphate

buffered saline (D-PBS, GIBCO #310-4190), and 2.5ml DMEM supplemented with 10% NuSerum (Collaborative Research #55000) were added to each plate. 2.5ml of the DNA/DEAE-dextran/chloroquine mix were added dropwise to each plate, the plates swirled to mix the DNA, and were returned to the incubator. After 4 hours in the incubator, the supernatant was aspirated from the cells and the cells were washed once with 5ml D-PBS. The cells were shocked for 3 minutes by the addition of 5ml of 10% dimethylsulfoxide (DMSO) in D-PBS at room temperature. The DMSO was aspirated from the cells and the cells were washed with 5ml D-PBS. 14ml of DMEM/10% FBS/2mM L-glutamine/1%P-S were added to each plate and the plates were returned to the incubator.

Three days post-transfection the medium was removed from the plates, pooled, and stored at -20°C. The cells were harvested, pooled, and seeded into 4 150cm^2 tissue culture flasks two with 40ml DMEM/10% NuSerum and two with 40ml DMEM/10% FBS/2mM L-glutamine. The medium was collected and the cells refed at 7, 10, and 14 days. In this way a total of 125ug of humanized 1308F antibody was accumulated in 310ml of medium supplemented with FBS and 85ug in 240ml of medium supplemented with NuSerum.

Example 5

Transfections of COS 1 cells with PSJ66

48 hours prior to transfection, 5 100mm tissue culture dishes were seeded with 1.5 x 10^6 cells per dish in 12ml DMEM, 10% FBS, 2mM L-glutamine, 1% penicillin-streptomycin (P-S, GIBCO #600-5070). The day of the transfection, 125ug of the plasmid pSJ66 were ethanol precipitated and aseptically resuspended in 1.0 ml Tris-Buffered-Saline. The resuspended DNA was added dropwise, with mixing, to 4.0ml of DMEM containing 1mg/ml DEAE-dextran (Pharmacia #17-0350-01) and 250uM chloroquine (Sigma #C6628). The medium was removed from the COS1 cells in the 100mm dishes and the cells were washed once with Dulbecco's phosphate buffered saline (D-PBS, GIBCO #310-4190), and 2.5ml DMEM supplemented with 10% NuSerum

(Collaborative Research #55000) were added to each plate. 2.5ml of the DNA/DEAE-dextran/chloroquine mix were added dropwise to each plate, the plates swirled to mix the DNA, and were returned to the incubator. After 4 hours in the incubator, the supernatant was aspirated from the cells and the cells were washed once with 5ml D-PBS. The cells were shocked for 3 minutes by the addition of 5ml of 10% dimethylsulfoxide (DMSO) in D-PBS at room temperature. The DMSO was aspirated from the cells and the cells were washed with 5ml D-PBS. 14ml of DMEM/10% FBS/2mM L-glutamine/1%P-S were added to each plate and the plates were returned to the incubator.

Three days post-transfection the medium was removed from the plates, pooled, and stored at -20°C. The cells were harvested, pooled, and seeded into 4 150cm^2 tissue culture flasks two with 40 ml DMEM10% NuSerum and two with 40 ml DMEM10% FBS/2mM L-glutamine. The medium was collected and the cells refed at 7, 10, and 14 days. In this way a total of 190ug of humanized 1308F antibody was accumulated in 310ml of medium supplemented with FBS and 120ug in 240ml of medium supplemented with NuSerum.

The concentration of humanized 1308F antibody secreted from the Cos1 cells into the medium was determined using a capture ELISA. Goat anti-human IgG Fc coated onto 96 well plates was used to capture the humanized antibody. Peroxidase conjugated goat anti-human whole IgG developed with a chromogenic substrate was then used to detect the bound antibody. A purified human IgG1/Kappa preparation was used to calibrate the assay.

Example 6

Neutralization of RSV with humanized 1308F

METHODS:

RSV was neutralized with either humanized 1308F from Cos cell supernatant or purified 1308F murine monoclonal antibody. This was done by incubating 50 plaque-forming units of RSV with serial 2-fold dilutions of antibody for 1.0 hour at 37°C. Confluent monolayers of Hep2 cells in 24 well panels were infected with 100μl

of antibody treated virus, untreated control virus, and mock infected controls. Incubated for 1.5 hours at 37°C, humidified , and 5% CO_2 and overlayed with 1.5mL EMEM, 1% FBS, and 1% methyl cellulose. Cells were fixed and stained with glutaldehyde and crystal violet on day 4. Plaques were counted in triplicate wells and plotted as percent neutralization. The results shown in Figure 6 indicate that both the purified murine 1308F monoclonal and the humanized 1308F monoclonal antibody at 5 to 10 ng per well yield similar 50% reductions in RSV plaques.

Example 7

Generation of a CDR-grafted A-site antibody 1129

Poly-A+ RNA was purified from a lysate of 2 x 107 murine 1129 hybridoma cells using oligo-dt cellulose. First strand CDNA was made from 1 ug pA+ RNA using random hexamer primers and AMV reverse transcriptase" 1ug pA+ RNA, 50mM Tris-HCl pH 8.5, 8mM Mg_2Cl, 30mM KCl, 1 mM dithiothrietol, 1 mM dNTP's, 25 units of placental ribonuclease inhibitor, 33uM random hexamer and 10 units of AMV reverse transcriptase for one hour at 42°C. The cDNA from the 1129 VL region was amplified by PCR using oligonucleotides SJ41 and SJ11, see Table 1. cDNA from the 1129 VH region was similarly amplified using oligonucleotides SJ42 and SJ10, see Table 1.

TABLE 1

SJ10
AGCGGATCCAGGGGCCAGTGGATAGAC

SJ11
GATGGATCCAGTTGGTGCAGCATC

SJ41
CACGTCGACATTCAGCTGACCCAGTCTCCA

SJ42
CGGAATTCAGGTIIAICTGCAGIAGTC(A,T)GG
{I = deoxy-Inosine}

SJ53
CCCAAGCTTGGTCCCCCCTCCGAACGTG

SJ154
GGCGTCGACTCACCATGGACATGAGGGTCC(C/T)CGCTCAGC

SJ155 (H1129L CDR 1)
GTCACCATCACTTGCAAGTGCCAGCTGAGTGTAGGTTACATGCACTGGTACC
AGCAG

SJ157 (H1129L CDR 3)
GCAACTTATTACTGCTTTCAGGGGAGTGGGTACCCATTCACGTTCGGAGGGG
GG

SJ168
GTGACCAACATGGACCCTGCTGATACTGCCAC

SJ169
CCATGTTGGTCACTTTAAGGACCACCTGG

SJ170
CCAGTTTACTAGTGTCATAGATCAGGAGCTTAGGGGC

SJ171
TGACACTAGTAAACTGGCTTCTGGGGTCCCATCAAGG

PCR conditions

0.5uL of 1st strand cDNA, 10mM Tris-HCl pH8.3, 50mM KCl, 1.5mM Mg2Cl, 0.2mM dNTP's, 0.001 % gelatin, 1 uM each primer, 1 ng DNA template and 2.5u AmpliTaq(TM) DNA polymerase (Perkin Elmer - Cetus). 94° 1 minute, 55° 2 minutes, 72° 2 minutes in Perkin Elmer 480 thermocycler for 25 cycles. The resulting DNA fragment(s) were then extracted once with phenol/chloroform (1/1), precipitated with 2.5 volumes of ETOH, resuspended in the appropriate restriction endonuclease buffer and digested with restriction endonucleases to produce cohesive ends for cloning. The resulting fragments were then separated by electrophoresis on a 1 % agarose gel. After staining the gel with ethidium bromide the fragments were excised and purified from the agarose by freezing and extraction in the presence of phenol.

The fragments were then digested with restriction endonucleases EcoR1 and BamH1 and cloned into plasmid pUC18. The inserts were

then sequenced by the dideoxynucleotide chain termination method using modified T7 DNA polymerase (Seqeunase, US Biochemical). The translated sequences were compared to human antibody protein sequences. The VL was found to be most homologous to the Kl02 light chain and the VH was found to be most homologous to the Cor VH region. The 1129 Fv region was then modeled by substitution of the residues from the 1129 VL and VH sequence into the coordinates of corresponding residues in the crystal structure the MCPC603 antibody. Residues were identified as being integral to the folded structure or solvent exposed by visual inspection of the model.

Several residues which were integral and which were different in the mouse and human sequences were left as the mouse residue in order to maintain the integrity of the Fv and thus the binding site. Such residues were 31,83,113, and 116 on the VH and 47 in the VL region. The resulting sequences are shown in figures 7 and 8.

The designed humanized 1129 VH was constructed using synthetic oligonucleotides SJ147-SJ153 (Figure 9) which were combined using PCR. The products of this PCR were then digested with Ncol and Sacl and cloned into pladmid vector pSJ40 which is a pUC18 derivative in which an out of frame lacZ1 segment is restored in frame as a fusion to an in-frame V region segment when such a segment is inserted as an Ncol-Sacl fragment. A plasmid containing an insert in which 5 mutations were clustered in a single 50 bp region was then subjected to repair of these changes using recombinant PCR and the primers SJ168 and SJ169, see Table 1.

The VL was generated by site directed mutagenesis of the humanized 1308F light chain gene. Oligonucleotides SJ155, see Table 1, (CDR1), and SJ157 (CDR3) were used to separately mutagenize the H1308L gene. Mutagenesis was carried out using T7 DNA polymerase on uracil containing single stranded DNA templates generated in E.

coli strain BW313 (dut-,ung-) and subsequently transformed into E. coli strain DH5 (dut+,ung+). The two mutants were combined and CDR2 introduced by recombinant PCR using oligonucleotides SJ170, SJ154, see Table 1, (5'end) and SJ171, SJ53, see Table 1, (3'end). The CDR-grafted VH and VL genes were placed into pSJ60 (see Example 3) and pSJ61 (see Example 3), respectively as Ncol-Sacl fragments in place of the H1308F Vregion segments resulting in plasmids pSJ81 and pSJ105. In addition the murine VH and VL cDNA segments were similarly joined to human C-Gammal and CKappa respectively to generate expression vectors pSJ75 and pSJ84.

Example 8

Hul129 Transient Expression

COS1 cells (ATCC CRL1650) were maintained in a humidified 5% CO_2 incubator in 75 CM^2 tissue culture flasks in Dulbecco's Modified Eagle Medium (DMEM, GIBCO #320-1965) supplemented with 10% fetal bovine serum (FBS, GIBCO #200-6140) and 2mM L-glutamine (GIBCO #320-5030) and passed at a split ratio of 1:20 just prior to reaching confluence.

Transfections were carried out according to the method of McCutchan and Pagano (J. Nat. Can. Inst. 41: 351-356, 1968) with the following modifications. Twenty four hours prior to transfection 100 mm tissue culture dishes (Corning # 25020) were seeded with 2 x 106 COS1 cells per dish in 14 ml DMEM, 10% FBS, 2mM L-glutamine. The day of the transfection 10 ug of the Hul129 heavy chain plasmid (pSJ81, from Example 7 were combined with 10 ug of the Hul129 kappa light chain plasmid pSJ105, from Example 7, the DNA was ethanol precipitated and aseptically resuspended in 1.0 ml Tris-Buffered-Saline. The resuspended DNA was added dropwise, with mixing, to 4.0 ml of DMEM containing 1 mg/ml DEAE-dextran (Pharmacia #170350-01) and 250 uM Chloroquine (Sigma #C6628). The medium was removed from the COS1 cell dishes, the cell monolayers were washed once with 10 ml Dulbecco's phosphate buffered saline

(D-PBS, GIBCO #310-4190), and 2.5 ml DMEM supplemented with 10% NuSerum (Collaborative Research #55000) and 2mM L-glutamine were added to each plate. 2.5 ml of the DNA/DEAEdextran/chloroquine mix were added dropwise to each plate, the plates were swirled to mix the DNA, and returned to the incubator. After an eight hour DNA adsorption period the plates were removed from the incubator and the supernatant was aspirated from the plates. The cells were shocked by the addition of 5 ml of 10% DMSO in D-PBS per plate for 3 minutes at room temperature, after which the DMSO was aspirated from the cells and the cells were washed once with 5 ml D-PBS. 15 ml DMEM, 10% NuSerum, 2mM L-glutamine (production medium) were added to each plate and the plates were returned to the incubator.

Seventy two hours post-transfection the conditioned medium was harvested from the plates and stored at -20°C, andl 5 ml production medium was added to the plates and the plates were returned to the incubator. Ninety six hours later the medium was collected from the plates and stored at 20°C.

Example 9

Quantitation of Hul129

Quantitation of the Hul129 IgG1 antibody secreted into the medium by the COS1 cells was performed using a sandwich type ELISA. In brief, Nunc Maxisorp Immunoplates (Nunc #439454) were coated with 50 ul/well of 0.5 ug/ml goat anti-human IgG Fc (Cappel #55071) in 0.1 M sodium bicarbonate pH 9.6 for 3 hours at room temperature. The wells were washed three times with 0.01 M sodium phosphate pH 7.4, 0.15 M NaCl, 0.1 % Tween 20 (PBS-T). Nonspecific protein binding to the plate was blocked by treatment of the wells with 200 ul/well of 3% (w/v) nonfat dry milk in PBS for 30 minutes at room temperature. A purified human IgG1 kappa standard (Sigma #1-3889) was made up at 100 ng/ml in PBS-T and serially diluted 1:2 to 1.56 ng/ml, and 50 ul of each were added to duplicate wells of the assay plate. COS1 cell supernatants were diluted in PBS-T and duplicate

50 ul samples were added to the plate. After an one hour room temperature incubation the wells were evacuated and washed three times with PBS-T. To detect the presence of bound Hul 129 antibody, horseradish peroxidase conjugated affinity purified goat anti-human IgG (whole molecule, Cappel #3601-0081) was diluted 1:1 000 in PBS-T and 50 ul was added to each well of the assay plate and incubated at room temperature for one hour. The plate was washed three times with PBS-T and 100 ul of the chromogenic substrate TMBlue (TSI #TM102) was added to each well. The plate was incubated at room temperature in the dark for ten minutes and the reaction was stopped by the addition of 50 ul per well of 4.5 M H_2SO_4. The plate was read at 450 nm using a Molecular Devices Vmax microplate reader, and data analysis was performed using Softmax software (Molecular Devices) running on an IBM P/S2 model 80 computer.

During the first seventy two hours of production the COS1 cells produced 0.06ug/ml Hu1129, for a total of 0.9ug. In the next ninety six hours of production the COS1 cells produced 0.99ug/ml Hu1129, for a total of 14.85ug.

Example 10

Hu1129 Binding Assay

Binding assays of the Hu1129 were performed in a capture ELISA, essentially as for the quantitation ELISA, but with the following changes. Plates were coated with the Mul 331 antibody at 0.5ug/well, the wells were blocked with 3% non-fat milk in PBS-T, and 50ul of RSV infected HEP2 cell lysate was added to each well and incubated at room temperature for 1 hour. The remainder of the assay was carried out as for the quantitation assay starting with the addition of diluted samples to the wells. Results were analyzed as a double reciprocal plot of OD vs antibody concentration from which an apparent Kd for the Hl129 molecule of

0.7nM was determined compared to 10nM for the M1129HuGamma1,Kappa antibody.

RSV neutralization assays on H1129 and ch1129 antibody were performed according to the following procedure:

1. Unwrap 96 well Costar cell culture plates in hood.

2. Warm Growth Medium (GM) to 37 C.

3. Thaw MA104 cells at 37 C. Dilute to ~150,000 cells per mL with GM. Mix cells and dispense 200 μl per well.

4. Culture cells 37 C, 5% CO_2, and humidified overnight before infection.

5. Dilute RSV Stock to 10,000 pfu per mL in Maintenance Medium (MM).

6. Mix equal volume of Antibody diluted in MM with equal volume of diluted RSV. Incubate at 37 C, 5% CO_2, and humidified for 1.0 h before infection.

7. Infect replicate wells of MA104 cells with 200 μl of the Antibody and Virus mixture. Infect replicate wells with virus and mock infected controls.

8. Wrap the plates in cellophane and incubate at 37 C, 95% humidity, and 5% CO_2 for 5 days.

9. <u>ELISA for RSV:</u> Aspirate each well; add 100 μl 80% Acetone/PBS (vol./vol.) and incubate at room temperature 30 minutes.

10. Aspirate each well and air dry for 30 minutes on the grill of a laminar flow hood.

11. Wash 4 times with PBS, 0.05%Tween 20.

12. Add 100 μl of monoclonal antibody to RSV F-protein to each well. Incubate for 1.0 h at 37 C.

13. Wash 4 times with PBS, 0.05%Tween 20.

14. Add 100 μl of anti-murine antibody goat serum-horse radish peroxidaze conjugate to each well. Incubate for 1.0 h at 37 C.

15. Wash 4 times with PBS, 0.05%Tween 20.

16. Add 100 μl of a freshly prepared 1:1 mixture of ABTS and peroxide to each well. Incubate at room temperature until the optical density (405 nm) of the virus control is 5 to 10 times that of the mock infected controls.

Appendix:

Growth Medium (GM): Minimum Essential Medium (Eagle) with
Earle's BSS,
2mM glutamine,
Eagle's non-essential amino acids 0.1 mM final,
Fetal bovine serum 10% (v/v),
Penicillin 50 units/ml,
Streptomycin 50 mcg/ml

Maintenance Medium (MM): as above with serum reduced to 1 to 2%.

MA104 cell stocks are grown up in T150 flasks with Growth Medium. Stocks are frozen at 3 x 10^6 cells per 1.8 mL vial in 10% DMSO and Growth Medium. Stored in a LN_2 refrigerator.

RSV stocks: are grown up in MA104 (monkey kidney) or Hep 2 cells in T150 flasks. Add ~0.2ml (~100,000 pfu) virus stock per confluent T150. Adsorption for 1.0 h at room temperature. Then add 20 mL maintenance medium with 1% fetal bovine serum. Incubate 4-5 days at 37 C. Collect cells just before 100% cpe by scraping. Spin down cells; remove all but 10 mL of supernatant. Freeze (dry ice-ethanol bath) thaw cell pellet, vortex, re-freeze, and store virus stock in LN2 refrigerator.

ELISA Antibody Buffer: PBS, 0.05%Tween 20 (w/v), 2.0% goat serum (v/v) and 0.5 % gelatin (w/v).

RSV F Protein Antibody: Chemicon Mab 858-1 anti-RSV fusion protein diluted ~1: 5000 in ELISA Antibody Buffer.

Anti-Murine Serum.: Fisher horse radish peroxidase conjugated to goat anti-mouse IgG (Heavy Chain Specific) diluted ~1: 4000 in ELISA Antibody Buffer.

The results are shown in Figure 10, and indicate 25ng/mi achieved 50% neutralization in this assay while 45ug/ml of the ch1129 antibody was required for 50% neutralization in this experiment. Over a series of 6 separate assays the mean 50% neutralization value for H1129 was 17ng/ml. As a control and to compare potency we also assayed a polyclonal human IgG preparation made from the plasma of individuals with high neutralizing titers for RSV. This preparation, termed RSVig (lot#4), gave a mean 50% neutralization value of 2.3ug/ml over 3 experiments. Thus the H1129 is 100-fold more potent in this assay as the enriched polyclonal preparation.

Example 11

Kinetic Analysis of Humanized RSV Mabs by BlAcoreTM

The kinetics of interaction between humanized RSV Mabs and the RSV F protein was studied by surface plasmon resonance using a Pharmacia BlAcoreTM biosensor. A recombinant baculovirus expressing a C-terminal truncated F protein provided an abundant source of antigen for kinetic studies. The supernatant, which contained the secreted F protein, was enriched approximately 20-fold by successive chromatography on concanalvalin A and Q-sepharose columns. The pooled fractions were dialyzed against 10 mM sodium citrate (pH 5.5), and concentrated to approximately 0.1 mg/ml. An aliquot of the F-protein (100 ml) was amine-coupled to the BlAcore sensor chip. The amount immobilized gave approximately 2000 response units (Rmax) Of signal when saturated with either H1129 or H1308F. This indicated that there was an equal number of "A" and "C" antigenic sites on the F-protein preparation following the coupling procedure. Two unrelated irrelevant Mabs (RVFV 4D4 and CMV H758) showed no interation with the immobolized F protein. A typical kinetic study involved the injection of 35 ml of Mab at varying concentrations (25-300 nM) in PBS buffer containing 0.05% Tween-20 (PBS/Tween). The flow rate was maintained at 5 ml/min, giving a 7 min binding phase. Following the injection of Mab, the flow was exchanged with PBS/Tween buffer for 30 min for determining the rate of dissociation. The sensor chip was regenerated between cycles with a 2 min pulse of 10 mM HCl. The regeneration step caused a minimal loss of binding capacity of the immobilized F-protein (4% loss per cycle). This small decrease did not change the calculated values of the rate constants for binding and dissociation.

The affinity of the various Mabs for binding to the F protein was calculated from the ratio of the first order rate constant for dissociation to the second order rate constant for binding (K_d =

k_{diss}/k_{assoc}). The value for k_{assoc} was calculated based on the following rate equation:

(1) $\mathbf{dR/dt = k_{assoc}[Mab]R_{max} - (k_{assoc}[Mab] + k_{diss})R}$

where R and Rmax are the response units at time t and infinity, respectively. A plot of dr/dt as a function of R gives a slope of (k_{assoc}[Mab] + k_{diss})- Since these slopes are linearly related to the [Mab], the value k_{assoc} can be derived from a replot of the slopes versus [Mab]. The slope of the new line is equal to k**assoc**. Although the value of k**diss** can be extrapolated from the Y-intercept, a more accurate value was determined by direct measurement of k_{diss}. Following the injection phase of the Mab, PBS/Tween buffer flows across the sensor chip. From this point, [Mab] = 0. Equation (1) thus reduces to:

(2) $\mathbf{dr/dt = k_{dissr}}$ **or** $\mathbf{dR/R = k_{diss}dt}$

Integration of equation (2) gives:

(3) $\mathbf{\ln(R_0/R_t) = k_{diss}t}$

where R_0/R_t) are the response units at time 0 (start of dissociation phase) and t, respectively. Lastly, plotting $\ln(R_0/R_t)$ as a function of t gives a slope of kdiss.

Kinetic Constants for RSV Mabs

Mab	ka(assoc) $M^{-1}sec^{-1}$	kd(dissoc) sec-1	$t_{1/2}$# (Hrs)	K_d (k_d/k_a) nM
CH1129	5.0×10^4	7.5×10^{-5}	2.6	1.5
H1129	4.9×10^4	6.9×10^{-5}	2.8	1.4
M1129	3.5×10^4	4.0×10^{-4}	0.48	11.4
M1308F	3.5×10^4	3.8×10^{-5}	5.1	1.1
H1308F	2.2×10^4	5.5×10^{-5}	3.5	2.5

References:

1. Hall, C.B., Doiuglas, R.G., Geiman, J.M. et al., N.Engl.J.Med. 293:1343, 1975.

2. Hall, C.B., McBride, J.T., Walsh, E.E. et al., N.Engl.J.Med. 308:1443, 1983.

3. Hall, C.B., McBride, J.T., Gala, C.L. et al., JAMA 254:3047, 1985.

4. Wald, E.R., et al., J.Pediat. 112:154, 1988.

5. Kapikian, A.Z., Mithcell, R.H., Chanock, R.M. et al., Am.J.Epidemiol. 89:405, 1969.

6. Prince, G.A., Hemming, V.G., Horswood, R.L. et al., Virus Res. 3:193, 1985.

7. Hemming, V.G., Prince, G.A., Horswood, R.L. et al., J.Infect.Dis. 152:1083, 1985.

8. Wright, P.F., Belshe, R.B., et al., Infect.Immun. 37:397, 1982.

9. Conrad, D.A., Christenson, J.C., et al., Peditr.Infect.Dis.J. 6:152, 1987.

10. LoBuglio, A.F., Wheeler, R.L., Trang, J. et al., Proc.Natl.Acad. Sci. 86:4220, 1989.

11. Steplewski, Z., Sun, L.K., Shearman, C.W. et al., Proc.Natl.Acad. Sci. 85:4852, 1988.

12. Boulianne, G.L., Hozumi, N., Shulman, M.J. Nature. 312:643, 1984.

13. Sun, L.K., Curtis, P., Rakowicz-Szulczynska, E. et al., Proc.Natl.Acad. Sci. 84:214, 1987.

14. Liu, A.Y., Mack, P.W., Champion, C.I., Robinson, R.R., Gene 54:33, 1987.

15. Morrison, S.L., Johnson, M.J., Hersenber, L.A., Oi, V.T. Proc.Natl.Acad. Sci. 81:6851, 1984.

16. Morrison, S.L. Science 229:1202, 1985.

17. Sahagan, B.G., Dorai, H., Saltzgaber-Muller, J. et al., J.Immunol. 137:1066, 1986.

18. Taked, S., Naito, T., Hama, K., Noma, T., Honjo, T., Nature 314:452, 1985.

19. Carson, D.A., Freimark, B.D., Adv. Immunol. 38:275, 1986.

20. Beeler, J.A., et al., J.Virol. 63:2941-2950, 1989.

21. Coelingh, et al., Virology, 143:569-582, 1985.

WHAT IS CLAIMED IS:

1. A human-murine chimeric antibody, comprising:

a human antibody containing at least one CDR from each of the variable heavy and variable light chains of a non-human monoclonal antibody against RSV.

2. An antibody as in Claim 1, wherein said murine monoclonal antibody is a neutralizing antibody against RSV.

3. An antibody as in Claim 1, wherein said murine monoclonal antibody is an antibody against RSV F protein.

4. An antibody as in Claim 3, wherein said murine monoclonal antibody is a neutralizing antibody against RSV F protein.

5. An antibody as in Claim 3, wherein:

said CDR comprises three complementarity determining regions from each of said variable heavy and variable light chains.

6. An antibody of Claim 5 wherein said murine antibody against RSV F protein is specific for antigenic site A of said protein.

7. A human antibody of Claim 5 wherein said murine antibody against RSV F protein is specific for antigenic site C of said protein.

8. A human antibody of Claim 7 wherein said murine antibody is MAb 1308F.

9. A human antibody as in Claim 8, wherein:

said three complementarity determining regions from said variable heavy chain of Mab 1308F comprise amino acid sequence Nos. 31 to 35, 47 to 60 and 99 to 106 and said three complementarity

determining regions from said variable light chain of MAb 1308F comprise amino acid sequence Nos. 24 to 34, 50 to 56 and 89 to 97.

10. A process for preventing or treating a respiratory syncytial virus infection in an animal comprising:

administering to said animal an effective amount of a human antibody which contains at least one CDR from each variable heavy chain and variable light chain, of at least one murine monoclonal antibody against respiratory syncytial virus F protein.

11. The process of Claim 10 wherein:

said CDR's have three complementarity determining regions from each of said variable heavy and variable light chains.

12. A composition for preventing or treating respiratory syncytial virus infection in an animal comprising:

(a) an effective amount of a human antibody which contains at least one CDR from each variable heavy and variable light chains of at least one murine monoclonal antibody against respiratory syncytial virus F protein, and

(b) an acceptable pharmaceutical carrier.

13. A process for preventing or treating a respiratory syncytial virus infection in an animal comprising:

administering to said animal an effective amount of a plurality of human antibodies which contain at least one CDR from each variable heavy and variable light chain of at least one murine monoclonal antibody against RSV F protein.

14. A human-murine chimeric antibody, comprising:

a human antibody containing at least one CDR from each of the variable heavy and variable light chains of a murine monoclonal antibody against RSV, where said murine antibody is MAb 1129.

15. An antibody as in Claim 14, wherein:

said CDR comprises three complementarity determining regions from each of said variable heavy and variable light chains.

16. A human antibody as in Claim 15, wherein:

said three complementarity determining regions from said variable heavy chain of Mab 1308F comprise amino acid sequence Nos. 31 to 35, 47 to 60 and 99 to 106 and said three complementarity determining regions from said variable light chain of MAb 1308F comprise amino acid sequence Nos. 24 to 34, 50 to 56 and 89 to 97.

17. A process for preventing or treating a respiratory syncytial virus infection in an animal comprising:

administering to said animal an effective amount of the human antibody of Claim 14.

18. A process for preventing or treating a respiratory syncytial virus infection in an animal comprising:

administering to said animal an effective amount of the human antibody of Claim 16.

19. A composition for preventing or treating respiratory syncytial virus infection in an animal comprising:

(a) an effective amount of the human antibody of Claim 14, and

(b) an acceptable pharmaceutical carrier.

20. A process for preventing or treating a respiratory syncytial virus infection in an animal comprising:

administering to said animal an effective amount of the composition of Claim 19.

Abstract of the Disclosure

This invention relates to a human antibody which contains the one CDR from each variable heavy and variable light chain of at least one murine monoclonal antibody, against respiratory syncytial virus which is MAb1129 and the use thereof for the prevention and/or treatment of RSV infection.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: JOHNSON, L.

(ii) TITLE OF INVENTION: Human Murine Chimeric Antibodies Against Respiratory Syncytical Virus

(iii) NUMBER OF SEQUENCES: 49

(iv) CORRESPONDENCE ADDRESS:

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(E) COUNTRY: USA

(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 INCH DISKETTE

(B) COMPUTER: IBM PS/2

(C) OPERATING SYSTEM: MS-DOS

(D) SOFTWARE: Word Perfect 5.1

(vi) CURRENT APPLICATION DATA:

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(B) FILING DATE: December 23, 1991

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Olstein, Elliot M.

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(C) REFERENCE/DOCKET NUMBER: 469201-257

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 201-994-1700

(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 27 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AGCGGATCCA GGGGCCAGTG GATAGAC 27

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 17 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TGGATGGTGG GAAGATG 17

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 15 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCCAGTGGA TAGAC 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 16 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TACAGTTGGT GCAGCA 16

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 24 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATGGATCCA GTTGGTGCAG CATC 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 30 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CACGTCGACA TTCAGCTGAC CCAGTCTCCA 30

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 30 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGGAATTCAG GTNNANCTGC AGNAGTCWGG 30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 28 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CCCAAGCTTG GTCCCCCCTC CGAACGTG 28

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 39 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGCGTCGACT CACCATGGAC ATGAGGGTCC YCGCTCAGC 39

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 57 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTCACCATCA CTTGCAAGTG CCAGCTGAGT GTAGGTTACA TGCACTGGTA CCAGCAG 57

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 54 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GCAACTTATT ACTGCTTTCA GGGGAGTGGG TACCCATTCA CGTTCGGAGG GGGG 54

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 32 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GTGACCAACA TGGACCCTGC TGATACTGCC AC 32

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 29 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CCATGTTGGT CACTTTAAGG ACCACCTGG 29

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 37 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CCAGTTTACT AGTGTCATAG ATCAGGAGCT TAGGGGC 37

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 37 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TGACACTAGT AAACTGGCTT CTGGGGTCCC ATCAAGG 37

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 97 AMINO ACIDS
(B) TYPE: AMINO ACID
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
5 10 15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn
20 25 30

Ser Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
35 40 45

Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr
50 55 60

Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
65 70 75

Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala

95

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 117 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
                 5                  10                  15

Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys
                20                  25                  30

Asp Tyr Tyr Ile Tyr Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
                35                  40                  45

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asn Thr Val Phe
                50                  55                  60

Asp Pro Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser
                65                  70                  75

Thr Ser Thr Val Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp
                80                  85                  90

Thr Ala Val Tyr Tyr Cys Ala Tyr Tyr Gly Thr Ser Ser Phe Asp
                95                 100                 105

Phe Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
               110                 115
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 117 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly
                 5                  10                  15
```

Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys
20 25 30

Asp Tyr Tyr Ile Tyr Trp Val Lys Gln Arg Pro Glu Gln Gly Leu
35 40 45

Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asn Thr Val Phe
50 55 60

Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ser Asp Thr Ser
65 70 75

Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp
80 85 90

Thr Ala Val Tyr Tyr Cys Ala Tyr Tyr Gly Thr Ser Ser Phe Asp
95 100 105

Phe Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110 115

(2) INFORMATION FOR SEQ ID NO:19

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 95 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val
5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
20 25 30

Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile

65 70 75

Ser Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
80 85 90

Tyr Asn Ser Tyr Ser
95

(2) INFORMATION FOR SEQ ID NO:20

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 107 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val
5 10 15

Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn
20 25 30

Arg Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
35 40 45

Leu Leu Ile Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile
65 70 75

Ser Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Leu Gln
80 85 90

Phe His Glu Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu
95 100 105

Ile Lys

(2) INFORMATION FOR SEQ ID NO:21

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 107 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr Val Ser Leu
5 10 15

Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn
20 25 30

Arg Tyr Leu Asn Trp Phe Gln Gln Lys Pro Gly Lys Ser Pro Lys
35 40 45

Thr Leu Ile His Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser
50 55 60

Arg Phe Ser Gly Ser Gly Ser Gly Gln Glu Tyr Ser Leu Thr Ile
65 70 75

Ser Ser Leu Glu Phe Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln
80 85 90

Phe His Glu Phe Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu
95 100 105

Ile Lys

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 117 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CCATGGACTG GACCTGGAGG GTCTTCTGCT TGCTGGCTGT AGCACCAGGT GCCCACTCCC 60

AGGTGCAGCT GGTGCAGTCT GGAGCTGAGG TGAAGAAGCC TGGAGCCTCA GTGAAGG 117

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 120 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CACTTCTTCG GACCTCGGAG TCACTTCCAA AGGACGTTCC GTAGACCTAA GTTGTAATTC 60
CTGATGATGT AAATGACCCA CGCTGTCCGA GGACCTGTTC CCGAGCTCAC CTACCCAACC 120

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 119 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGGCTCGAGT GGATGGGTTG GATTGACCCT GAGAATGGTA ATACTGTGTT TGACCGAAGT 60
TCCAGGGCAG AGTCACCATG ACCAGGGACA CGTCCACGAG CACAGTCTAC ATGGAGCTG 119

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 137 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GGTGCTCGTG TCAGATGTAC CTCGACTCGT CGGACTCTAG ACTCCTGTGC CGGCACATAA 60
TGACACGCAT GATGCCATGT TCGAGGAAAC TGAAGACCCC GGTTCCGTGG TGAGAGTGTC 120
ACTCGAGTAT TCCTAGG 137

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 106 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CCATGGACAT GAGGGTCCCC GCTCAGCTCC TGGGGCTCCT GCTGCTCTGG CTCCCAGGTG 60
CCAAATGTGA TATCCAGATG ACCCAGTCTC CTTCCACCCT GTCTGC 106

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 107 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTCAGAGGAA GGTGGGACAG ACGTAGACAT CCTCTGTCTC AGTGGTAGTG AACGTTCCGC 60
TCAGTCCTGT AATTATCCAT AAATTTGACC ATGGTCGTCT TTGGGCC 107

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 107 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAAAGCCCCT AAGCTCCTGA TCTATCGTGC AAACAGATTG GTAGATGGGG TCCCATCAAG 60
GTTCAGCGGC AGTGGATCTG GGACAGAATT CACTCTCACC ATCAGCA 107

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 116 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTCTTAAGTG AGAGTGGTAG TCGTCGGACG TCGGACTACT AAAACGTTGA ATAATGACGG 60
ATGTCAAAGT ACTCAAAGGC ATGTGCAAGC CTCCCCCCTG GTTCGAACTT TATTTT 116

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 123 AMINO ACIDS
(B) TYPE: AMINO ACID
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr
5 10 15

Gln Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser

20 25 30

Ser Ser Gly Met Cys Val Gly Trp Ile Arg Gln Pro Pro Gly Lys
35 40 45

Ala Leu Glu Trp Leu Ala Asp Ile Glu Trp Asp Asp Asp Lys Asp
50 55 60

Tyr Asn Thr Ser Leu Asp Thr Arg Leu Thr Ile Ser Lys Asp Thr
65 70 75

Ser Lys Asn Gln Val Val Leu Thr Val Thr Asn Met Asp Pro Ala
80 85 90

Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Ile Thr Val Ile Pro Ala
95 100 105

Pro Ala Gly Tyr Met Asp Val Trp Gly Arg Gly Thr Pro Val Thr
110 115 120

Val Ser Ser

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 120 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr
5 10 15

Gln Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser
20 25 30

Thr Ser Gly Met Ser Val Gly Trp Ile Arg Gln Pro Ser Gly Lys
35 40 45

Ala Leu Glu Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp
50 55 60

Tyr Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr

65 70 75

Ser Lys Asn Gln Val Val Leu Lys Val Thr Asn Met Asp Pro Ala
80 85 90

Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Ser Met Ile Thr Asn Trp
95 100 105

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 120 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Gln Val Glu Leu Gln Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser
5 10 15

Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser
20 25 30

Thr Ser Gly Met Ser Val Gly Trp Ile Arg Gln Pro Ser Gly Glu
35 40 45

Gly Leu Glu Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp
50 55 60

Tyr Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr
65 70 75

Ser Ser Asn Gln Val Phe Leu Lys Ile Thr Gly Val Asp Thr Ala
80 85 90

Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Ser Met Ile Thr Asn Trp
95 100 105

Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
110 115 120

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 95 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val
                 5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
                20                  25                  30

Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
                35                  40                  45

Leu Leu Ile Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                50                  55                  60

Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile
                65                  70                  75

Ser Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln
                80                  85                  90

Tyr Asn Ser Tyr Ser
                95
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 106 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val
                 5                  10                  15

Gly Asp Arg Val Thr Ile Thr Cys Lys Cys Gln Leu Ser Val Gly
                20                  25                  30

Tyr Met His Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu
```

35 40 45

Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser
65 70 75

Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Phe Gln Gly
80 85 90

Ser Gly Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
95 100 105

Lys

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 106 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro
5 10 15

Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Gly
20 25 30

Tyr Met His Trp Tyr Gln Gln Lys Ser Ser Thr Ser Pro Lys Leu
35 40 45

Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Gly Arg
50 55 60

Phe Ser Gly Ser Gly Ser Gly Asn Ser Tyr Ser Leu Thr Ile Ser
65 70 75

Ser Ile Gln Ala Glu Asp Val Ala Thr Tyr Tyr Cys Phe Gln Gly
80 85 90

Ser Gly Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
95 100 105

Lys

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 63 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GCCTGAGCTC ACGGTGACCG TGGTCCCGCC GCCCCAGACA TCGAAGTAGC AGTTCGTGAT CAT 63

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 79 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTTGGTGACT TTAAGGACCA CCTGGTTTTT GGAGGTATCC TTGGAGATTG TGAGCCGGCT 60

CTTCAGCCAT GGATTATAG 79

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 89 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

GCGCCTTCCC TGGGGGCTGA CGAATCCAGC CTACACTCAT ACCAGAAGTG CTCAGTGAAA 60

ACCCAGAGAA GGTGGAGGTC AGTGTGAGG 89

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 70 NUCLEOTIDES

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CCAGGTCACC TTAAGGGAGT CTGGTCCTGC GCTGGTGAAA CCCACACAGA CCCTCACACT GACCTGCACC 60 70

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 78 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: Oligonucleotide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CAGCCCCCAG GGAAGGCCCT GGAGTCGCTT GCAGACATTT GGTGGGATGA CAAAAAGGAC TATAATCCAT CCCTGAAG 60 78

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 64 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: Oligonucleotide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GGTCCTTAAA GTGACCAACA TGGACCCTGC TGATACTGCC ACTTACTACT GTGCTCGGTC TATG 60 64

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 72 NUCLEOTIDES
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR
(ii) MOLECULE TYPE: Oligonucleotide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GGCGTCGACT CACCATGGAC TGGACCTGGA GGGTCTTCTG CTTGCTGGCT GTAGCACCAG GTGCCCACTC CC 60 72

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 7 AMINO ACIDS
(B) TYPE: AMINO ACID
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Thr Ser Gly Met Ser Val Gly
5

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 16 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asp Ile Trp Trp Asp Asp Lys Lys Asp Tyr Asn Pro Ser Leu Lys Ser
5 10 15

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 10 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Ser Met Ile Thr Asn Trp Tyr Phe Asp Val
5 10

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 10 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Lys Cys Gln Leu Ser Val Gly Tyr Met His
5 10

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Thr Ser Lys Leu Ala Ser
5

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 8 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Phe Gln Gly Ser Gly Tyr Pro Phe
5

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 6 AMINO ACIDS

(B) TYPE: AMINO ACID

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Ser Val Gly Tyr Met His
5

Figure 1

DESIGN OF CDR-GRAFTED ANTI-RSV F PROTEIN V_H

```
                   5                  10                  15                  20
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val  Human HV3 VH

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val  "CDR Grafted" VH
 *                  *                       *   *   *                   *               *
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala Leu Val Lys Leu  Murine 1308F VH

                   25                  30                  35                  40
Ser Cys Lys Ala Ser Gly Tyr Thr Phe Asn Ser Tyr Tyr Met His Trp Val Arg Gln Ala
                           *   *   *   *   *               *   *
Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Ile Tyr Trp Val Arg Gln Ala
                                                 CDR 1                 *       *
Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Ile Tyr Trp Val Lys Gln Arg

                   45                  50                  55                  60
Pro Gly Gln Gly Leu Glu Trp Met Gly Ile Ile Asn Pro Ser Gly Gly Ser Thr Ser Tyr
                           *       *       *       *   *       *       *   *
Pro Gly Gln Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asn Thr Val Phe
                    *                      CDR 2
Pro Glu Gln Gly Leu Glu Trp Ile Gly Trp Ile Asp Pro Glu Asn Gly Asn Thr Val Phe

                   65                  70                  75                  80
Ala Gln Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
 *   *
Asp Pro Lys Phe Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Thr Ser Thr Val Tyr
                           *   *   *   *       *               *   *       *
Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ser Asp Thr Ser Ser Asn Thr Ala Tyr

                   85                  90                  95                 100
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Tyr Tyr Gly
 *                         *
Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Tyr Tyr Gly
                                                                <<V  /     D
                  105                 110                 115
 -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -

Thr Ser Ser Phe Asp Phe Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
       CDR 3
Thr Ser Ser Phe Asp Phe Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
           /  J>>
```

Figure 2

DESIGN OF CDR-GRAFTED ANTI-RSV F PROTEIN VL

```
                    5                   10                  15                  20
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly Asp Arg Val Thr  - Human K102 VL

Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly Asp Arg Val Thr  - "CDR Grafted" VL
        *                       *   *   *   *       *       *
Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr Val Ser Leu Gly Glu Arg Val Thr  - Murine 1308F VL

                   25                  30                  35                  40
Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro
             *             *       *   *   *       *
Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Arg Tyr Leu Asn Trp Tyr Gln Gln Lys Pro
                                  CDR 1                         *
Ile Thr Cys Lys Ala Ser Gln Asp Ile Asn Arg Tyr Leu Asn Trp Phe Gln Gln Lys Pro


                   45                  50                  55                  60
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
                                *       *   *       *   *
Gly Lys Ala Pro Lys Leu Leu Ile Tyr Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser
          *             *           *           CDR 2
Gly Lys Ser Pro Lys Thr Leu Ile His Arg Ala Asn Arg Leu Val Asp Gly Val Pro Ser


                   65                  70                  75                  80
Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
                                  *       *   *                               *
Arg Phe Ser Gly Ser Gly Ser Gly Gln Glu Tyr Ser Leu Thr Ile Ser Ser Leu Glu Phe


                   85                  90                  95                 100
Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Tyr Asn Ser Tyr Ser  -   -   -
                                  *       *   *   *   *   *
Asp Asp Phe Ala Thr Tyr Tyr Cys Leu Gln Phe His Glu Phe Pro Tyr Thr Phe Gly Gly
 *       *   *   *                          CDR 3
Glu Asp Met Gly Ile Tyr Tyr Cys Leu Gln Phe His Glu Phe Pro Tyr Thr Phe Gly Gly
                                                    <<V  /  J>>

                   105
 -   -   -   -   -   -   -

Gly Thr Lys Leu Glu Ile Lys

Gly Thr Lys Leu Glu Ile Lys
```

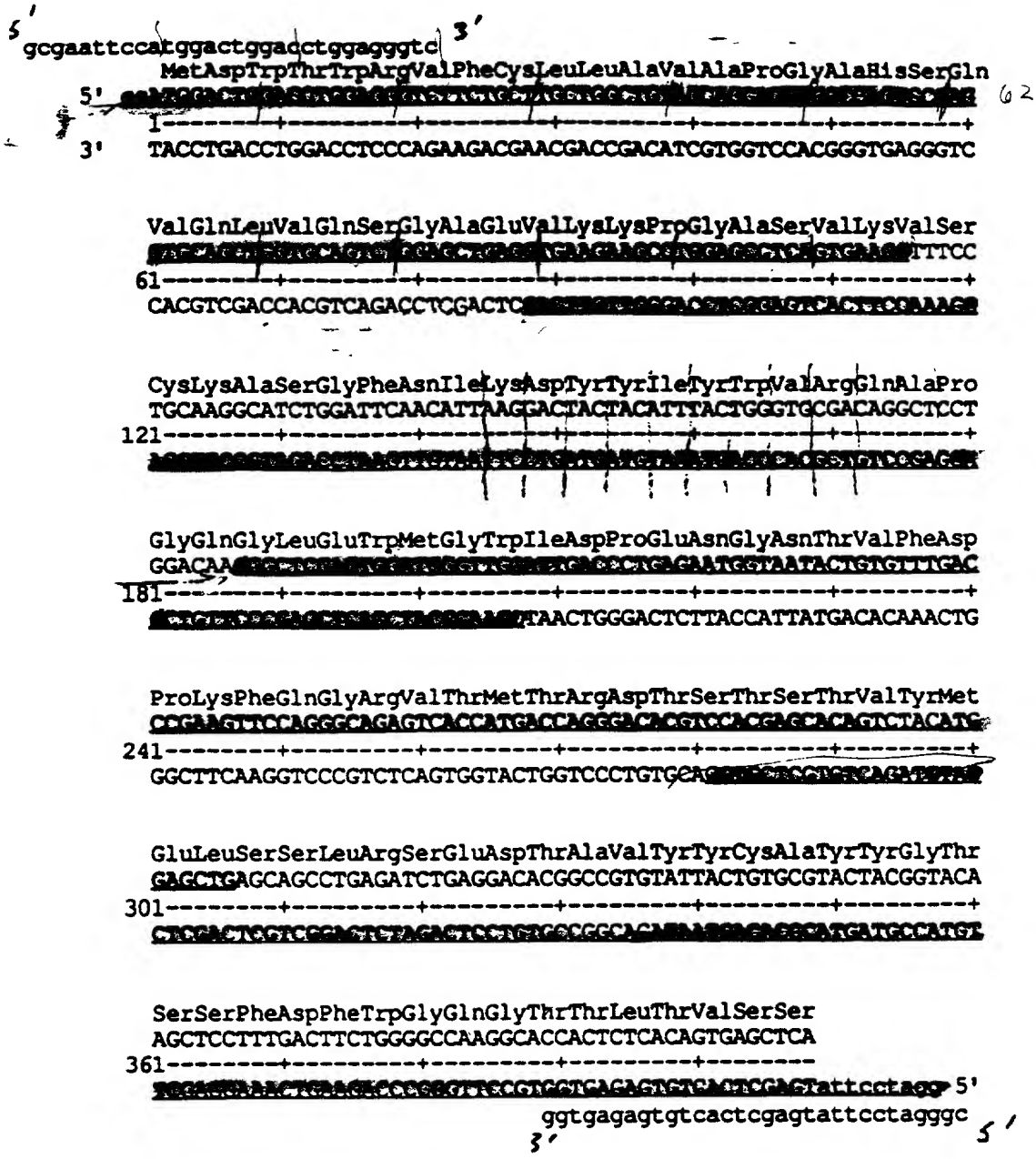

Figure 3. oligos used to make Hu1308 VH

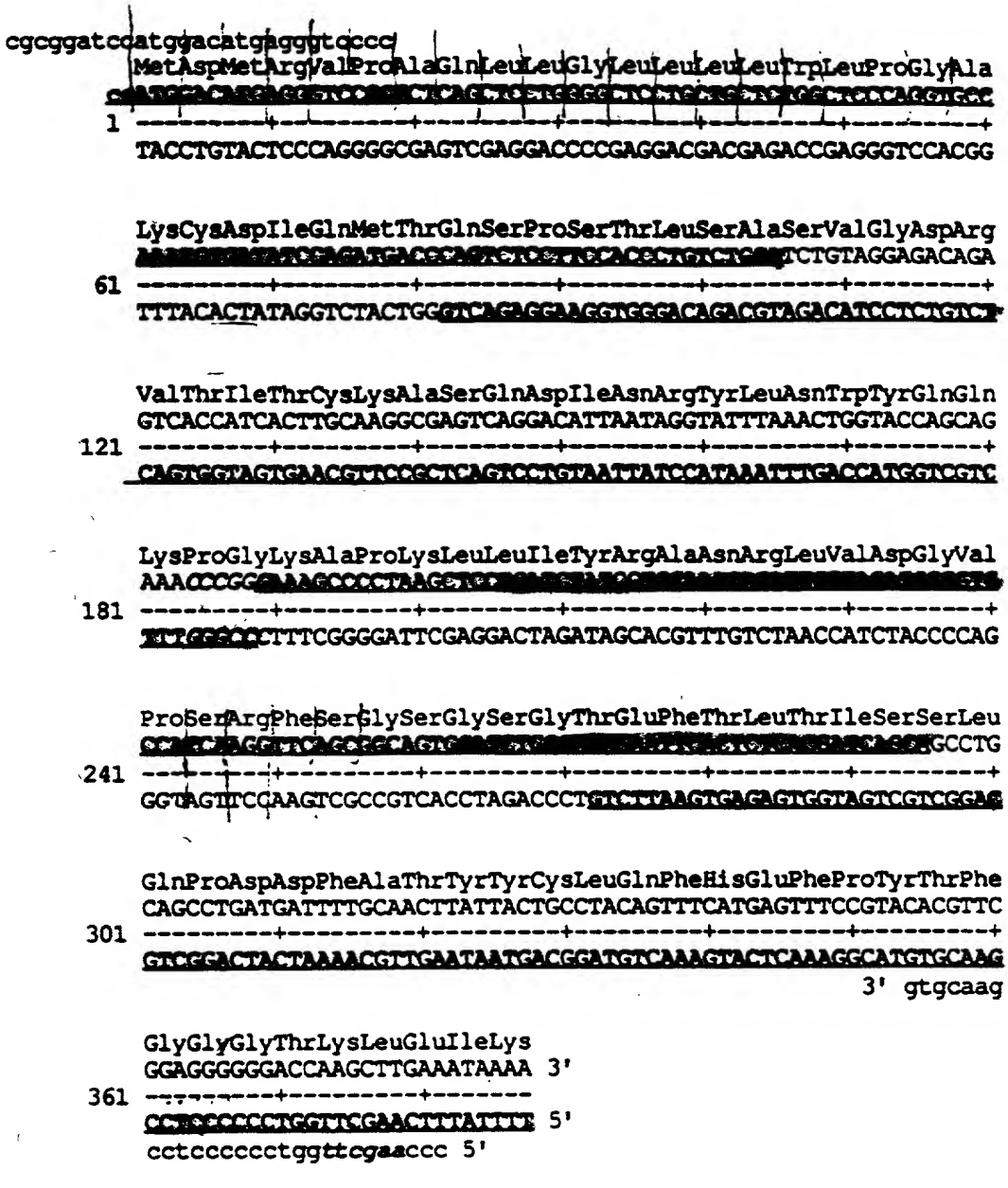

Figure 4. Oligos used to make Hd308 V_L

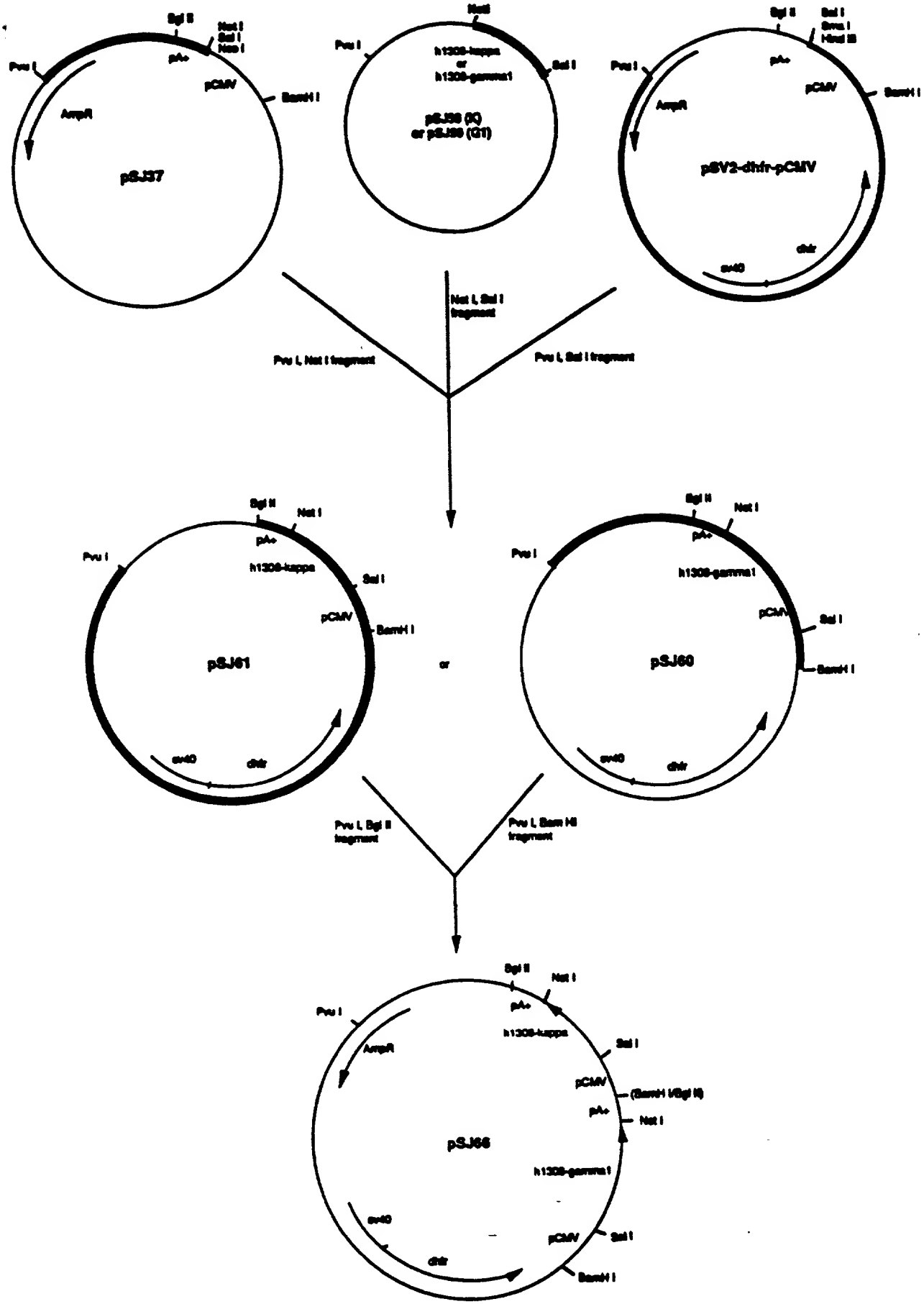

Fig 5. Construction of Humanized 1308 expression vectors

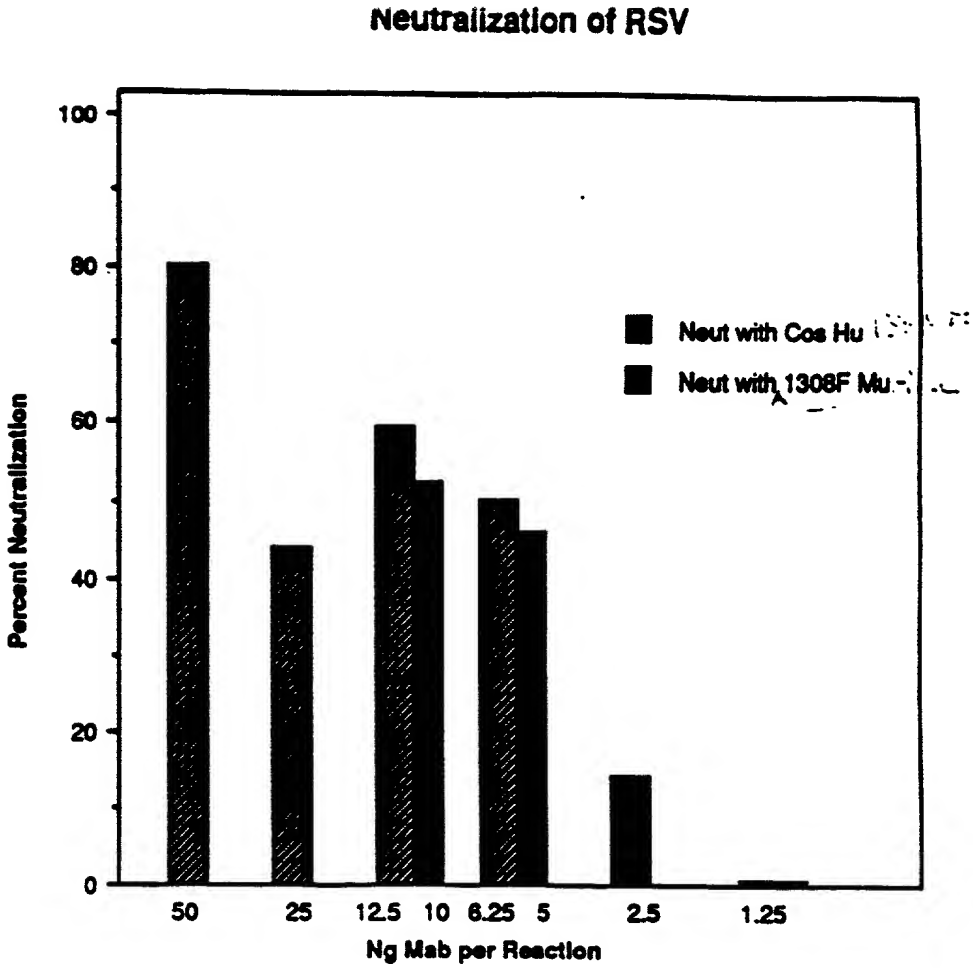
Neutralization of RSV
Percent Neutralization
100
80
60
40
20
0
50
25
12.5
10
6.25
5
2.5
1.25
Ng Mab per Reaction
Neut with Cos Hu
Neut with 1308F Mu

FIGURE 6

Design of Humanized VH for anti-RSV Mab 1129

```
                        5                   10                  15
   Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Ser  Human VH (Cor)
1  Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro (Ser) "Humanized" VH
                   *                   *   *   *   *    -
   Gln Val Glu Leu Gln Glu Ser Gly Pro Gly Ile Leu Gln Pro Ser  Murine 1129 VH


   Gln Thr Leu Thr Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser
16 Gln Thr Leu Thr Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser
                *
   Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu Ser


   Ser Ser Gly Met Cys Val Gly Trp Ile Arg Gln Pro Pro Gly Lys
     *       *                                 *           *
31 Thr Ser Gly Met Ser Val Gly Trp Ile Arg Gln Pro Pro Gly Lys
   Thr Ser Gly Met Ser Val Gly Trp Ile Arg Gln Pro Ser Gly Glu


   Ala Leu Glu Trp Leu Ala Asp Ile Glu Trp Asp Asp Asp Lys Asp
                                     *             *
46 Ala Leu Glu Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp
     *
   Gly Leu Glu Trp Leu Ala Asp Ile Trp Trp Asp Asp Lys Lys Asp


   Tyr Asn Thr Ser Leu Asp Thr Arg Leu Thr Ile Ser Lys Asp Thr
             *           *   *
61 Tyr Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr
   Tyr Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr


   Ser Lys Asn Gln Val Val Leu Thr Val Thr Asn Val Asp Pro Ala
                               *                               —
76 Ser Lys Asn Gln Val Val Leu Lys Val Thr Asn Val Asp Pro Ala
       *           *           *   *           *
   Ser Ser Asn Gln Val Phe Leu Lys Ile Thr Gly Val Asp Thr Ala


   Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Ile Thr Val Ile Pro Ala Pro Ala Gly
                                     *   *   *   *   *   *
91 Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Ser Met Ile Thr Asn Trp  -   -   -
   Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Ser Met Ile Thr Asn Trp  -   -   -


   Tyr Met Asp Val Trp Gly Arg Gly Thr Pro Val Thr Val Ser Ser
         *             *             *
106 Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
   Tyr Phe Asp Val Trp Gly Ala Gly Thr Thr Val Thr Val Ser Ser
```

Figure 7

DESIGN OF CDR-GRAFTED ANTI-RSV MAb 1129 V_L

```
                    5                  10                  15
Asp Ile Gln Met Thr Gln Ser Pro Ser Thr Leu Ser Ala Ser Val   - Human K102 VL
                 *                  *   *   *               *
Asp Ile Gln Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro   - Murine 1129 VL

                   20                  25                  30
Gly Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser
                                 *   *   *   *           *   *
Gly Asp Arg Val Thr Ile Thr Cys Lys Cys Gln Leu Ser Val Gly
      *   *              *          *   *   *   *      CDR 1
Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Gly

                   35                  40                  45
Ser Trp Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys
*   *   *
Tyr Met His   -   Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys

Tyr Met His   -   Trp Tyr Gln Gln Lys Ser Ser Thr Ser Pro Lys

                   50                  55                  60
Leu Leu Ile Tyr Asp Ala Ser Ser Leu Glu Ser Gly Val Pro Ser
      *               *   *       *
Leu Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ser
                          CDR 2
Leu Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Gly

                   65                  70                  75
Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile

Arg Phe Ser Gly Ser Gly Ser Gly Thr Glu Phe Thr Leu Thr Ile
                                    *   *   *   *
Arg Phe Ser Gly Ser Gly Ser Gly Asn Ser Tyr Ser Leu Thr Ile

                   80                  85                  90
Ser Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Gln Gln

Ser Ser Leu Gln Pro Asp Asp Phe Ala Thr Tyr Tyr Cys Phe Gln
          *       *   *       *
Ser Ser Ile Gln Ala Glu Asp Val Ala Thr Tyr Tyr Cys Phe Gln

                   95                 100                 105
Tyr Asn Ser Tyr Ser
 *   *   *   *
Gly Ser Gly Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
        CDR 3
Gly Ser Gly Tyr Pro Phe Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
```

<<V / J>>

Figure 8

Oligonucleotides for the construction of the Humanized 1129 VH gene segment

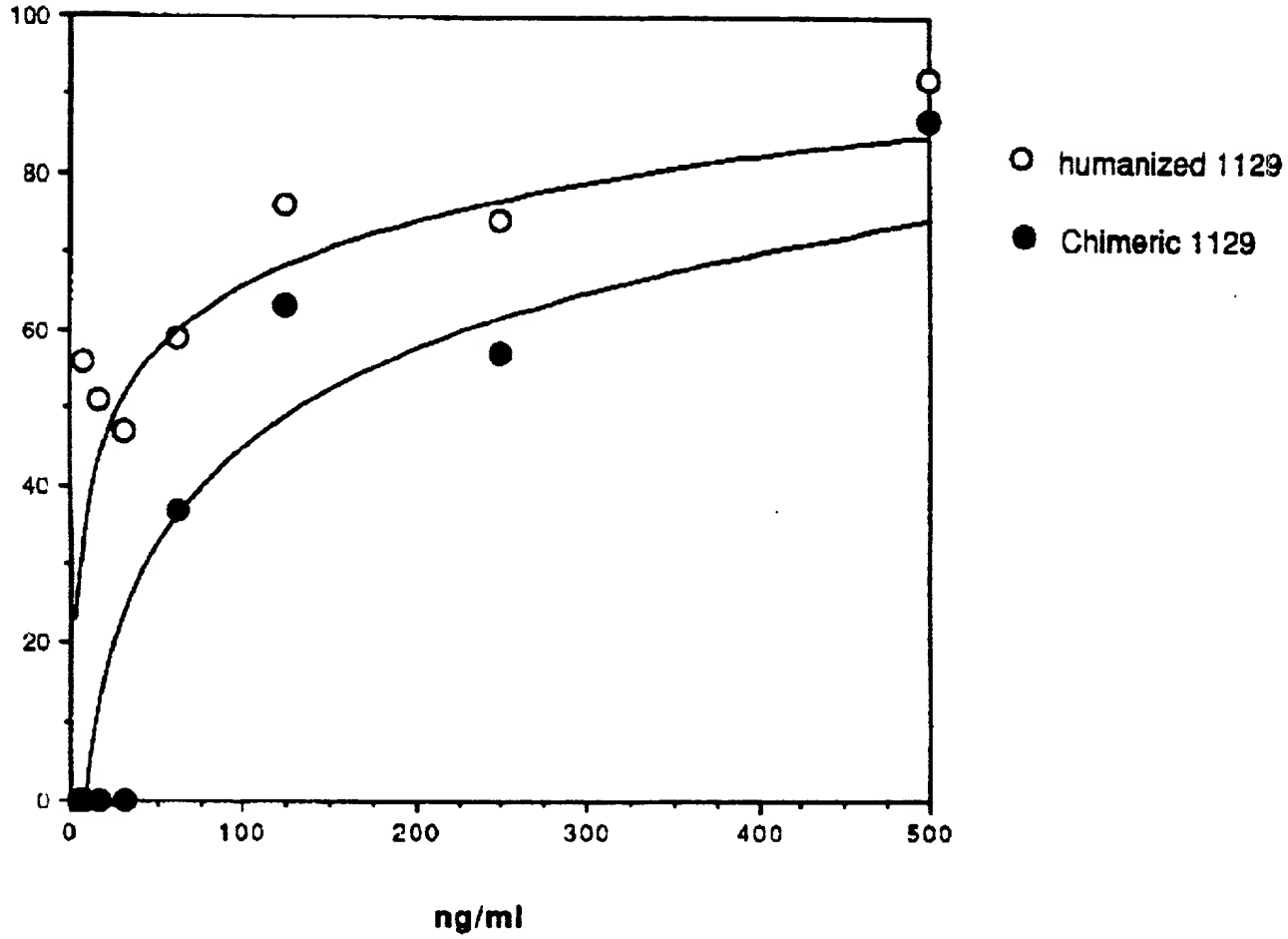
100
80
60
40
20
0
% Neutralization
0
100
200
300
400
500
ng/ml
humanized 1129
Chimeric 1129

Figure 10

PATENT07/FORMS

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My resident, post office address and citizenship are as stated below next to my name

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled HUMAN-MURINE CHIMERIC ANTIBODIES AGAINST RESPIRATORY SYNCYTICAL VIRUS

the specification of which is attached hereto unless the following box is checked:

___ was filed on ____________________ as Application Serial No. ______________ and was amended on ____________________ (if applicable).

I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATIONS

			Priority Claimed
None			
(NUMBER)	(COUNTRY)	(DAY/MONTH/YEAR FILED)	YES/NO
(NUMBER)	(COUNTRY)	(DAY/MONTH/YEAR FILED)	YES/NO
(NUMBER)	(COUNTRY)	(DAY/MONTH/YEAR FILED)	YES/NO

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose information which is material as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

07/813,372	Dec. 23, 1991	Pending
(Applicant Number)	(Filing Date)	(Status-patented, pending, abandoned
(Applicant Number)	(Filing Date)	(Status-patented, pending, abandoned

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office contracted therewith:

John N. Bain	Reg. No. 18,651
John G. Gilfillan III	Reg. No. 22,746
Elliot M. Olstein	Reg. No. 24,025
Raymond J. Lillie	Reg. No. 31,773
Charles J. Herron	Reg. No. 28,019
[illegible]	Reg. No. 25,3[illegible]
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Address all correspondence to Mr. Olstein
Carella, Byrne, Bain, Gilfillan,
Cecchi, Stewart & Olstein
6 [illegible] Farm Road
Roseland, New Jersey 07068

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and

further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full name of sole or first inventor (given name, family name) LESLIE SID JOHNSON
Inventor's signature _____ Date _____

Residence 13545 Ambassador Drive Citizenship U.S.A.
Germantown, Maryland 20874
Post Office Address same as above

Full name of second joint inventor, if any (given name, family name) _____
Second Inventor's signature _____ Date _____

Residence _____ Citizenship _____

Post Office Address _____

Full name of third joint inventor (given name, family name) _____
Inventor's signature _____ Date _____

Residence _____ Citizenship _____

Post Office Address _____

PATENT07/FORMS

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As a below named inventor, I hereby declare that:

My resident, post office address and citizenship are as stated below next to my name

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled HUMAN-MURINE CHIMERIC ANTIBODIES AGAINST RESPIRATORY SYNCYTICAL VIRUS

the specification of which is attached hereto unless the following box is checked:

x was filed on 8/15/94 as Application Serial No. 08/290,592
and was amended on _____ (if applicable).

I hereby state that I have reviewed and understood the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATIONS

			Priority Claimed
None			
(NUMBER)	(COUNTRY)	(DAY/MONTH/YEAR FILED)	YES/NO
(NUMBER)	(COUNTRY)	(DAY/MONTH/YEAR FILED)	YES/NO
(NUMBER)	(COUNTRY)	(DAY/MONTH/YEAR FILED)	YES/NO

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07/813,372	Dec. 23, 1991	Pending
(Applicant Number)	(Filing Date)	(Status-patented, pending, abandoned
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Raymond J. Lillie	Reg. No. 31,778
Charles J. Herron	Reg. No. 28,019
William Squire	Reg. No. 25,378
Gregory D. Ferraro	Reg. No. 36,134

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Address all correspondence to Mr. Olstein
Carella, Byrne, Bain, Gilfillan,
Cecchi, Stewart & Olstein
6 Becker Farm Road
Roseland, New Jersey 07068

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and

further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Full name of sole or first inventor (given name, family name) LESLIE SID JOHNSON
Inventor's signature [signature] Date 11/22/94

Residence 13545 Ambassador Drive, Germantown, Maryland 20874 Citizenship U.S.A.

Post Office Address ~~same as above~~

Full name of second joint inventor, if any (given name, family name) ______________

Second Inventor's signature ______________ Date ______________

Residence ______________ Citizenship ______________

Post Office Address ______________

Full name of third joint inventor (given name, family name) ______________
Inventor's signature ______________ Date ______________

Residence ______________ Citizenship ______________

Post Office Address ______________